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| 1635 | |

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Please find below and/or attached an Office communication concerning this application or proceeding.

# Office Action Summary

| Application No. | Applicant(s) |
|---|---|
| 09/448,613 | McCray et al |
| **Examiner** | **Art Unit** |
| Richard Schnizer | 1635 |

-- *The MAILING DATE of this communication appears on the cover sheet with the correspondence address* --

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136 (a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1) [X] Responsive to communication(s) filed on *Mar 4, 2003* .

2a) [ ] This action is **FINAL**. 2b) [X] This action is non-final.

3) [ ] Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

**Disposition of Claims**

4) [X] Claim(s) *1-8, 10-39, 41-44, and 46-70* is/are pending in the application.

4a) Of the above, claim(s) *57-59, 61, and 62* is/are withdrawn from consideration.

5) [ ] Claim(s) _____ is/are allowed.

6) [X] Claim(s) *1-8, 10-39, 41-44, 46-56, 60, and 63-70* is/are rejected.

7) [ ] Claim(s) _____ is/are objected to.

8) [ ] Claims _____ are subject to restriction and/or election requirement.

**Application Papers**

9) [X] The specification is objected to by the Examiner.

10) [X] The drawing(s) filed on *Nov 22, 1999* is/are a) [X] accepted or b) [ ] objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11) [ ] The proposed drawing correction filed on _____ is: a) [ ] approved b) [ ] disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12) [ ] The oath or declaration is objected to by the Examiner.

**Priority under 35 U.S.C. §§ 119 and 120**

13) [ ] Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a) [ ] All b) [ ] Some* c) [ ] None of:

1. [ ] Certified copies of the priority documents have been received.
2. [ ] Certified copies of the priority documents have been received in Application No. _____ .
3. [ ] Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

*See the attached detailed Office action for a list of the certified copies not received.

14) [X] Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

a) [ ] The translation of the foreign language provisional application has been received.

15) [ ] Acknowledgement is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

**Attachment(s)**

1) [X] Notice of References Cited (PTO-892)
2) [ ] Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) [ ] Information Disclosure Statement(s) (PTO-1449) Paper No(s). ___
4) [ ] Interview Summary (PTO-413) Paper No(s). ___
5) [ ] Notice of Informal Patent Application (PTO-152)
6) [ ] Other:

## DETAILED ACTION

Applicant's Appeal Brief was received and entered as Paper No. 20 on 3/4/03.

After further consideration, finality of the previous Office Action is withdrawn in favor of the following non-final Action.

Claims 9, 40, and 45 were canceled by amendment in Paper No. 14, filed 3/18/02.

Claims 1-8, 10-39, 41-44, and 46-70 remain pending in the application.

The restriction requirement issued in Paper No. 7 required Applicant to elect a virus, a gene, a disease, and a permeabilizing agent. Applicant elected without traverse in Paper No. 9 the species of invention comprising retroviruses, membrane channels, cystic fibrosis, and a hypotonic solution with a chelator. Claims 13-25, 57-59, 61, and 62 were withdrawn from further consideration in Paper No. 10 pursuant to 37 CFR 1.142(b) as being drawn to nonelected species, there being no allowable generic or linking claim.

In light of Applicant's arguments in the paragraph bridging pages 7 and 8 of the Appeal Brief, and the discussion that occurred at the PTO's Appeals Conference for this application, the species election requirement is withdrawn with respect to claims 1-8, 10-39, 41-44, and 46-52, and 68-70, but is maintained with respect to claims 53-67. Further, claim 53 will be considered to be a linking claim for four distinct methods of treating diseases as follows:

claims 53-56, 60, and 63-67 drawn to methods of treating cystic fibrosis;

claims 53-59, and 64-67, drawn to drawn to of treating epithelial cancer;

claims 53-56, 60, 61, and 64-67, drawn to drawn to methods of treating epithelial surfactant protein B deficiency; and

claims 53-56, 62, and 64-67, drawn to methods of treating alpha-1-antitrypsin deficiency.

Upon the allowance of the linking claim(s), the restriction requirement as to the linked inventions shall be withdrawn and any claim(s) depending from or otherwise including all the limitations of the allowable linking claim(s) will be entitled to examination in the instant application. Applicant(s) are advised that if any such claim(s) depending from or including all the limitations of the allowable linking claim(s) is/are presented in a continuation or divisional application, the claims of the continuation or divisional application may be subject to provisional statutory and/or nonstatutory double patenting rejections over the claims of the instant application. Where a restriction requirement is withdrawn, the provisions of 35 U.S.C. 121 are no longer applicable. *In re Ziegler*, 44 F.2d 1211, 1215, 170 USPQ 129, 131-32 (CCPA 1971). See also MPEP § 804.01.

Consistent with Applicant's original species election, claims 53-56, 60, and 63-67 are under consideration in this Office action, limited to methods of treating cystic fibrosis by increasing epithelial transmembrane permeability by treatment with a hypotonic solution and a chelating agent. Claims 57-59, 61, and 62 stand withdrawn from consideration as drawn to a non-elected invention.

Subsequent to rejoining claims 13-25, claims, 1-8, 10-39, 41-44, 46-56, 60, and 63-70 are under consideration in this Office Action.

Claims limited to methods of treating cystic fibrosis by increasing epithelial transmembrane permeability by treatment with a hypotonic solution and a chelating agent were found to be novel and non-obvious over the prior art. In accordance with MPEP 803.02, the Office extended the search to a second species of invention, *i.e.* methods of treating cystic fibrosis by increasing epithelial transmembrane permeability by treatment with polylysine, and contacting the epithelial tissue with a retrovirus encoding a membrane channel. Claims reciting this species have been found to be obvious over the prior art for the reasons given below. Claim 66, requiring increasing proliferation of diseased epithelial cells by treatment of a proliferative agent is free of the art of record in all embodiments.

***Rejections Withdrawn***

After further consideration the rejection under 35 USC 102 of claims 1, 2, 4, 6-8, 26-31, and 48-52 over Halbert is withdrawn in favor of the new grounds of rejection set forth below.

***Specification***

The specification is objected to because "peracellular" is misspelled at page, line 10; "Corect" is misspelled at page 66, line 2; "perfommed" is misspelled at page 69, line 13; "legal" is substituted for "beta galactosidase" at page 74, line 9, and "rioted" is substituted for "noted" at page 74, line 23; "vito" is misspelled at page 79, line 12. The heading for Example 4 at page 60,

line 2 and 3 is objected to because it indicates that integrating vectors can correct the CF defect in differentiated cells in vivo, but no evidence to this effect is presented in Example 4.

***Claim Objections***

Claim 1 is objected to because it is ungrammatical. Insertion of the article "a" prior to the word "composition" is recommended.

Claim 11 is objected to because it is ungrammatical. Insertion of the article "an" prior to the word "ion" is recommended.

***Claim Rejections - 35 USC § 112***

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 2-5, 10-33, 35-37, 53-56, 60, and 63-67 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 2-5 and 26-33 are indefinite because they recite "said epithelial tissue" without antecedent basis.

Claims 10-33, 36, and 37 are indefinite because they depend from canceled claim 9. For the purpose of examination under 35 USC 102, these claims have been treated as if they depended from claim 1.

Claims 26-31 are ambiguous. These claims are drawn to methods of increasing the susceptibility of epithelial cells to viral infection wherein a viral infection step is required in the method. However, the claims do not make clear when in the method the viral infection step should take place. As a result, it is unclear whether the recited viral infection step is part of a method of increasing susceptibility of cells to a *subsequent* viral infection, or whether the recited viral infection step is the viral infection to which the tissue has been rendered susceptible.

Claims 35 and 36 are indefinite because they recite "said proliferative factor" without antecedent basis.

Claims 53-56, 60, and 63-67 are indefinite because they recite "said therapeutic polypeptide" without antecedent basis.

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-8, 10-37, 53-56, 60, 63-67, and 70 are rejected under 35 U.S.C. 112, first paragraph, because the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with these claims.

The instant invention is directed to improving the efficiency of gene transfer to epithelial

cells by increasing the transepithelial permeability of an epithelial sheet. Recent publications, as well as the specification, indicate that receptors for adenovirus, adeno-associated virus, and retroviruses tend to be sequestered on the basolateral surface of lung epithelial cells, and are therefore not available to bind viruses delivered via the lumen of the lung. The essence of the instant invention is to increase transepithelial permeability thereby either allowing access to viral receptors or allowing receptor independent infection. The specification teaches working examples in which adenovirus, retrovirus (MuLV), lentivirus (FIV), and adeno-associated virus infection of epithelial tissue is increased by treatment of epithelial tissue with permeabilizing agents. See e.g. page 58, lines 11-28; page 59, lines 20-27; page 72, lines 9-16; page 74, lines 4-18; and paragraph bridging pages 80 and 81.

1. Pertinent to claims 1-8 and 10-37, the specification, while enabling for methods of increasing the susceptibility of epithelial tissue to infection with viral vectors, comprising the step of increasing the transepithelial permeability of the epithelium by treating the epithelium with an epithelium permeabilizing agent, does not reasonably enable methods of increasing the susceptibility of epithelial tissue to infection with non-vector viruses, or for methods of increasing the susceptibility of epithelial tissue to infection with viral vectors by treating the epithelium with any and all tissue permeabilizing agents.

This portion of the rejection relates to the breadth of the claim term "viruses", and to the breadth of the claim term "tissue permeabilizing agent."

The specification teaches at page 1, lines 9-11 that the invention relates to methods of increasing susceptibility of epithelial tissue to infection by viruses or viral vectors. One of skill in the art, after reading the specification, would interpret the term "viral vector" in the context of the invention to be limited to recombinant viral expression vectors carrying foreign genes. The scope of the term "viruses" is much broader and includes wild type viruses. The specification does not teach any purpose for increasing the susceptibility of epithelia to wild type virus infection, none was found in the prior art of record, and none is readily apparent. Because there is no apparent reason for increasing the susceptibility of infection to wild type viruses, and because the specification fails to teach any, one of skill in the art would not know how to use the invention, and the scope of viruses for which the specification is enabling is limited to "viral vectors".

The specification does not limit the scope of tissue permeabilizing agents embraced by the instant claims. As a result the claims embrace a genus of permeabilizing agents that will not function in the invention. For example, dimethyl sulfoxide (DMSO) is recognized in the art as a tissue permeabilizing agent. See e.g. Allen et al (DEVELOPMENT, (1990 Apr) 108 (4) 623-34), abstract. Yet DMSO actually decreases transepithelial permeability in cultured Caco-2 epithelia (Mariadason et al (Am. J. Physiol. (1997) 272: G705-G712) see abstract), and would not function in the invention to increase transepithelial permeability. It is suggested that the scope of the permeabilizing agent should limited to epithelial tissue permeabilizing agents.

2. Pertinent to claims 26-34, the specification, while enabling for methods of increasing susceptibility of epithelial tissue to infection with viral expression vectors comprising the steps of increasing the transepithelial permeability of the epithelium by treating the epithelium with an epithelium permeabilizing agent, and delivering a viral expression vector to the permeabilized epithelium, does not reasonably enable methods of increasing susceptibility of epithelial tissue to infection with viral expression vectors wherein the viral expression vector is not administered to epithelial tissue while it remains permeabilized. In other words, because permeabilization is transient, to be operable the invention must require administration of viral vectors to tissue while the tissue remains permeabilized. Evidence that permeabilization is transient can be found e.g. in Marano et al (Biochem. Biophys. Res. Comm. (1995) 209(2): 669-676), Li et al (BIOCHIMICA ET BIOPHYSICA ACTA, (1990 Dec 14) 1030 (2) 297-300), and Wong et al (J. Cell Biol. (1997) 1363(2): 399-409). Marano teaches that the increase in transepithelial permeability obtained by treatment of epithelia with TNF- alpha does not occur until 2 hours after treatment, and then subsides within 4-6 hours. See paragraph bridging pages 673 and 674. Li teaches that the increased epithelial tight junction permeability obtained by treatment of the epithelium with FCCP occurred almost immediately, but dissipated by 2 hours. See abstract. Wong teaches that the increase in paracellular transepithelial permeability obtained by treatment with an occludin peptide begins to reverse immediately upon removal of the peptide. See e.g. Fig 6A on page 406. Because increases in transepithelial permeability within the context of the invention are reversible, the claims should reflect that virus infection should occur during the period when the

transepithelial permeability is increased. It is suggested that the claims should be amended to require viral vector administration to permeabilized epithelium.

3. Pertinent to claims 48 and 49, the specification, while enabling for methods of redistributing viral receptors from the basolateral to the apical side of an epithelial cell of an epithelial tissue, comprising contacting the tissue with an agent that increases transepithelial permeability, fails to reasonably enable the broader method redistributing viral receptors from one cell to another by increasing transepithelial permeability of for methods of redistributing viral receptors by means not requiring contacting the cells with an agent that increases transepithelial permeability.

The claims embrace methods of redistributing viral receptors on epithelial cells, but do not limit the type of redistribution, so the receptors may be redistributed anywhere within a cell or even between cells. The specification provides guidance only with regard to redistributing receptors from the basolateral to the apical sides of cells. The prior art of record does not teach methods of redistributing receptors between epithelial cells by increasing intraepithelial permeability. Because the specification fails to teach that which is missing from he prior art, it is enabling only for the type of redistribution for which it provides guidance, i.e. redistribution of viral receptors from the basolateral to the apical side of an epithelial cell of an epithelial tissue.

The method as claimed requires a step of increasing transepithelial permeability, but recites no means by which transepithelial permeability may be increased. Hence, the means by

which transepithelial permeability may be increased are unlimited. The specification teaches only means which involve directly contacting the epithelial tissue with an agent that causes an increase in transepithelial permeability. A review of the prior art did not reveal any other methods. Therefore the claim embraces methods which go beyond those taught in the specification or the prior art, and which would require undue experimentation in order to perform. This portion of the rejection can be overcome by requiring that the epithelial sheet must be contacted with an agent that increases transepithelial permeability.

4. Claim 70 is drawn to a method of transforming epithelial cells with a viral vector. The issue for consideration here is the meaning of the word "transforming". The specification does not define this term, however it s a term of art that has different meanings in different contexts. In the context of prokaryotic cells, it is generally used to refer to the process of delivering a nucleic acid such that a phenotype is altered. In the context of viral infection of eukaryotic cells, "transformation" refers to malignant transformation of cells. See attached definition from *The Dictionary of Cell and Molecular Biology* (retrieved from http://www.mblab.gla.ac.uk/~julian/Dict.html on 5/9/03). The specification does not limit the scope of the viral vector that can be used in the claimed method, and it is clear to those of skill in the art that not all viral vectors, particularly those designed for gene therapy, should cause malignant transformation of cells. For these reasons one of skill in the art could not obtain malignant transformation of cells with the recombinant viral vectors disclosed in the specification.

This portion of the rejection can be overcome by substituting the word "transducing", or the word "infecting", for the word "transforming."

5. Claims 53-56, 60, 63-67 contain subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

Claims 53-56, 60, 63-67 are drawn to methods of treating any epithelial tissue disease. The elected embodiment is limited to treatment of cystic fibrosis (CF), so in order to enable the elected invention, the specification must teach how to treat CF by administration to epithelial cells *in vivo* of a retroviral vector encoding a therapeutic gene. The scope of the term "treating" is considered to be very broad, and embraces results ranging from the expression of a functional CFTR chloride channel in a patient to completely curing the patient.

With respect to the generic claims embracing treatment of any and all epithelial tissue diseases, it is noted that at time the invention was made successful implementation of gene therapy protocols was not routinely obtainable by those skilled in the art. This is reflected by three recently published reviews. Orkin (Report and Recommendations of the Panel to Assess the NIH Investment in Research on Gene Therapy, 1995) teaches that "significant problems remain in all basic aspects of gene therapy. Major difficulties at the basic level include shortcomings in all current transfer vectors and an inadequate understanding of the biological interaction of these vectors with the host" (page 1, item 3). Orkin teaches that problems exist in delivering nucleic

acid sequences to the appropriate target cell or tissue and achieving the appropriate level of expression within that cell or tissue (page 9).Verma et al (Nature 389: 239-242, 1997) teach that "there is still no single outcome that we can point to as a success story (p. 239, col 1). The authors state further, "Thus far, the problem has been the inability to deliver genes efficiently and to obtain sustained expression" (p.239, col. 3). Anderson (Nature 392:25-30, 1998) confirms the unpredictable state of the art, stating that "there is still no conclusive evidence that a gene-therapy protocol has been successful in the treatment of human disease" (p. 25, col. 1) and concluding, "Several major deficiencies still exist including poor delivery systems, both viral and non-viral, and poor gene expression after genes are delivered" (p.30). As discussed more fully below, the specification fails to provide the guidance that is missing from the prior art with regard to providing therapy for the elected disease of CF, much less for the scope of epithelial tissue diseases embraced by claims 53-62 and 64-67. As such one of skill in the art would have to perform undue experimentation to practice the invention commensurate in scope with the claims.

The remainder of the discussion pertains to the specific embodiment of CF gene therapy.

The prior art teaches a method of providing cystic fibrosis transmembrane conductance regulator to airway epithelial cells of a cystic fibrosis patient comprising combining cationic polypeptide polymers such as poly-L-lysine or histone with adenoviral particles containing a transgene encoding cystic fibrosis transmembrane conductance regulator, wherein said transgene is expressed and a functional chloride channel is produced in the airway epithelial cells of said patient. See e.g. Welch (US Patent 5,962,429, claim 13). The prior art does not teach any

method of obtaining clinically relevant therapeutic benefit from methods of in vivo CFTR gene delivery.

At the time of the invention, the treatment of CF through the administration of therapeutic genes was not routinely practiced with success by those of skill in the art due to in part to poor gene transfer efficiency and expression as discussed above. In addition to the basic problems of gene delivery and expression and the lack of adequate models, one of the most important barriers to gene therapy of CF is an incomplete understanding of the pathophysiology of the disease. This is manifested in the lack of information regarding the appropriate target cells for gene delivery. Rosenfeld and Collins (Chest 109:241-252, 1996) teach that it is unclear exactly which cells should receive [gene therapy]", stating that "[t]he difficulty in determining which cells to target relates to an inability to draw parallels between the normal pattern of CFTR expression and the development of CF in lung disease. In normal individuals, the surface epithelium of small airways expresses very low levels of CFTR, while the submucosal glands found exclusively in large airways express much higher levels. In contrast, in CF, the most important pathologic consequences occur first in the small airways with alveolar damage as a consequence. Little if any clinically significant disease ever occurs where the submucosal glands are found." Boucher (TIG 12(3): 81-84, 1996) notes that this issue is relevant to strategies for vector delivery because while the superficial epithelium of airways can be reached by lumenal vector delivery, the submucosal glands may require systemic administration. See page 1 of reprint, column 2, last sentence of first full paragraph. Rosenecker (Eur. J.. Med. 23(3): 149-156, 3/1998) teaches that "[t]opical

administration of gene transfer vectors to airways is impeded by surface fluid, mucus plugging the airway lumen, and the ciliated apical surface of epithelial cells" and that the submucosal glands are inaccessible for topically applied formulations. Thus systemic delivery via the blood stream is indicated. See page 152, column 2, lines l-15 of second full paragraph. The instant specification fails to address systemic delivery in the treatment of CF, focusing instead on lumenal delivery. So, if it turns out that transfection of submucosal glands is required for the treatment of CF, then the specification has failed to provide an enabling disclosure because it provides insufficient guidance as to how to deliver retroviral vectors to cells of the submucosal glands. Further evidence of the unpredictability of CF gene therapy comes from Wilson (J. Clin. Invest. 96(2547-2554, 12/1995) who teaches that the program of expression of CFTR in the lung is extraordinarily complicated, and that the effect of omitting submucosal glands from treatment is unknown. Wilson also notes that vector targeting and gene expression are currently nonspecific, resulting in ectopic and unregulated expression of CFTR. See page 2548, first full paragraph.

Because it is unknown what cell types need to be transfected in order to treat CF, it is also unclear how many cells must be transfected and what level of gene expression is required in order to achieve therapy. This is readily apparent from a review of the literature both before and after the filing date of the instant application. See Rosenfeld and Collins (1996, first full paragraph of column 1 on page 243); Boucher (TIG 1.2(3): 81-84, 1996, page 81, paragraph bridging columns 2 and 3); Alton and Geddes (J. R. Soc. Med 90 Suppl 31: 43-46 1997); Davies (Mol. Med Today 4(7): 292-299, 7/1998, page 294, column 2, lines 20-28); Boucher (J. Clin.

Invest. 103(4): 441-445 2/1999); and Flotte (Chest 120: 124S-131S, 2001, page 124S, column 2 second full paragraph). The level of expression required for therapy is also unknown because the relationship between abnormal ion transport and pathophysiology of CF is incompletely understood. Briefly, the molecular problem responsible for CF is a defect in a chloride ion transporter known as CFTR. One hypothetical explanation for the progress of the disease depends on a failure to transport chloride ions, leading to abnormal absorption of sodium ions by the epithelium. This leads to dehydration and thickening of the mucus in the lungs, which in turn leads to a variety of pathophysiological outcomes including inflammation, repeated infections, and decreasing pulmonary function. Alternatively, the defect in CFTR could somehow affect the actual composition of mucus in the lung, resulting in the recognized pathologies. See Wilson (1995) paragraph bridging pages 2547 and 2548. Thus a primary focus of treatment is the restoration of chloride ion transport. Boucher (1999) teaches that it is likely that the percentage of epithelial cells requiring functional correction to restore normal chloride ion transfer in vivo may well exceed 10%, and advises that the simplest strategy to assure efficacy is to mimic the normal pattern of in vivo expression by achieving gene expression in 100% of lung epithelial cells. See paragraph bridging pages 441 and 442, page 442 column l, lines 25-30, and 42- 45. The instant specification fails to teach how to achieve this level of gene delivery.

Boucher concludes that a one or two order of magnitude increase in in vivo gene transfer efficiency, above that observed in clinical trials, will be required for therapeutic relevance in CF treatment. See page 444, column 2, first sentence of second full paragraph. Clinical studies have

shown success in partially correcting chloride ion transport, however Alton and Geddes (1997) teach that it is unknown whether the chloride or sodium defect associated with CF is the more important error to correct, and that the degree of correction needed for clinical benefit of these defects is unknown. See page 45, lines 7-10 of first full paragraph. Furthermore, Davies (1998) teaches that if normalization of sodium ion transport is required for therapeutic effect, then the levels of gene transfer observed to date will be inadequate because correction of sodium ion transport has not been achieved in the majority of preclinical and clinical studies. See page 294, column 2, lines 22-28. Rosenfeld (1996) indicates that although restoration of chloride conductance in monolayer cells is achieved by transfection of 5-7% of the cells, normalization of sodium ion reabsorption will require transfection of a much higher percentage of cells. See page 243, column 1, lines 15-1 8. This view is also expanded on by Jiang et al (Eur. J. Hum. Genet. (1998) 6(1): 12-31) who teach that "it is now clear that the pathophysiology of CF airways disease is far more complex than can be solely attributed to altered chloride permeability. For example, in addition to functioning as a chloride channel, CFTR has been implicated in the regulation of other apical membrane conductance pathways through interaction with the amiloride sensitive epithelial sodium channel and the outwardly rectifying chloride channel. Superimposed on this functional diversity of CFTR is a highly regulated pattern of CFTR expression in the lung. This heterogeneity occurs at both the level of CFTR expression within different cell types in the airway and the anatomical location of these cells in the lung." See abstract. Jiang goes on to indicate that because of this complexity, it is unclear what endpoint should be measured in clinical

trials, indicating in particular that it is unclear that the correction of chloride ion transport is relevant as a measure for the efficacy for reversing disease pathophysiology. See page 26, column 2, lines 10-25.

The claimed invention comprises a step of increasing transepithelial permeability in order to increase viral transduction. Similarly Johnson et al (J. Virol. (1998) 72(11): 8861-8872) evaluated the effect of permeabilizing lung epithelia on gene transfer with VSV-G pseudotyped MuLV retroviral vectors. Mice lungs were treated with a permeabilizing agent, and retroviral gene transduction efficiency of 6.1% was obtained. Non-permeabilized controls achieved only 0.09% transduction. However, even though the authors noted that this efficiency was within the range predicted to correct Cl- transport in CF patients (and was greater than that disclosed in the instant application), the authors concluded that "further refinements in improving host modification to increase access of vector to the proliferative pool of cells in the airway environment of the CF lung and a better understanding of the barriers to efficient transduction of all proliferating cells will be required if these pseudotyped MuLV vectors are to play a significant role in gene therapy approaches for CF lung disease." See page 871, column 1, last sentence.

For these reasons it was apparent at the time of the invention that the practice of gene therapy of CF was highly unpredictable. Shortly after the application was filed, Boucher (1999) summarized the state of the art by stating that "despite an impressive amount of research in this area, there is little evidence to suggest that an effective gene transfer approach for the treatment of CF lung disease is imminent." Notably, this review was published after the Jonson (1998)

paper on which Boucher was a coauthor. Subsequently Rodgers et al (Eur. Respir. J (2001): 17: 1314-1321) reviewed the estate of the art of CF therapy in general and concluded that "gene therapy as a treatment for CF has yet to be realized." See abstract. In 2002, O'Dea et al (Current Gene Therapy (2002) 2: 173-181) reviewed the state of the art of CF gene therapy and stated that "efficient delivery and stable transfer of genes such as CFTR to human lung epithelium [has] yet to be accomplished." See sentence bridging columns 1 and 2 on page 173. Finally, earlier this year, almost five years after the effective filing date of the application, Ferrari et al reviewed the state of CR gene therapy (Clin Exp. Immunol. (2003) 132: 1-8). While the review focused on immunological barriers to lung gene therapy, the authors also noted that "current levels of gene transfer efficiency are too low to result in clinical benefit, largely as a result of the barriers faced by gene transfer vectors within the lung [citation omitted]. A complex series of extracellular barriers such as mucus, mucociliary clearance and glycocalyx proteins are known to limit the access of gene transfer agents to lung cells."

The specification teaches working examples in which normal rabbit lungs were treated with EGTA in vivo in order to either permeabilize the epithelium, or cause receptor redistribution, prior to infection with retroviral vectors. From 2.5% to 4.8% percent of rabbit lung epithelial cells were transfected. See page 73, lines 19-26, and page 74, lines 10-12, and 25-27. However, the teachings of the those of skill in the art at the time of the invention indicate that transfection of at least 6-10% of epithelial cells would be required in order to restore normal chloride ion transport in vivo. See Johnson et al (of record C40). However, as noted by Boucher (1999), this

estimate is based on in vitro assays using monolayer epithelial cells which were highly connected by gap junctions. This allows chloride ions from over-corrected cells to diffuse to uncorrected cells. Boucher indicates that it is likely that the number of gap junctions in vivo is less than that in the in vitro monolayer model, so the minimum number of cells which must be transfected in vivo may well exceed 10%. See page 442, column 1, lines 5-29. As noted above, this issue is further complicated by the fact that it is not known what level, if any, of chloride ion transport will result in correction of the sodium ion transport defect, which may be more important in the pathology of the disease. See Alton and Geddes (1997) and Davies (1998), above. It is further noted that the rabbits used in these assays were normal, see page 73, line 23, and thus did not suffer from the accumulation of mucus associated with the CF in humans, which impedes vector access to the epithelium according to Rosenecker (1998, see page 152, column 2, lines 1-15 of second full paragraph). See also Davies (1998) page 292, column 2, lines 7-9 of first paragraph. For this reason, one of skill in the art could not reproduce in a CF patient the level of gene transfer observed in the rabbit model of the working example. Thus, even if one accepts that 6-10% transfection of epithelial cells is sufficient for treatment of CF, and it is clear that the art recognizes that this may be an extremely optimistic estimate, the instant specification has failed to teach how to achieve this level of transfection in a CF patient.

It is also noted that the scope of membrane channels which may be used in the invention is not limited to CFTR but encompasses all membrane channels. Even if the specification taught how to use the CFTR membrane channel to treat CF, which it does not, the specification does not

teach how to use any other membrane channel in the treatment of CF. The CFTR polypeptide is a chloride ion transporter which appears to be regulated by phosphorylation. See Rosenecker (1998, page 149, column 2, lines 11-14). One of skill in the art would not expect that a membrane channel designed for transport of other ions could be used to treat the disease, and the specification offers no guidance in this regard. For example, the specification fails to teach how to use the FOFI-ATPase/synthase, which comprises a membrane channel for hydrogen ions, in the treatment of CF. Furthermore, the specification fails to teach any example of a membrane channel that responds to the same regulatory signals as CFTR and in the same ways. Thus the specification has failed to teach how to restore appropriate cellular function using membrane channels other than CFTR, and one of skill in the art could not treat CF with such channels without undue experimentation.

In summary, while the specification teaches how to improve viral infection of epithelial cells in vivo, it fails to teach how to use the claimed methods and compositions to obtain therapy for CF. The specification fails to add to the teachings of the prior art with respect to the identification of the type of cells which must be transfected, the number of cells which must be transfected, or the level of expression which is required in order to obtain therapy of CF. It fails to teach whether or not CF therapy can be obtained by restoring only chloride ion transport, or if restoration of the sodium ion defect is required. It also fails to teach how to achieve correction of the sodium ion defect. Finally it fails to teach how to transfect the minimum number of cells which the prior art suggests will be required to obtain therapeutic benefit in CF. Furthermore,

gene therapy of CF is highly unpredictable because the prior art had established that the target cells for treatment, the characteristics of expression and required for therapy, and the nature of the defect which required correction were unknown.

Because the prior art teaches that it is not known which cells must be transfected with CFTR expression vectors in order to obtain therapeutic benefit in CF treatments, how many of these cells must be transfected, or what level of expression must be obtained to effect therapy; because the instant invention provides transfection of a lower percentage of cells than the minimum which is deemed necessary by those of skill in the art to correct the CF chloride ion transport defect; because it is unknown if correction of the chloride ion transport defect will result in any therapeutic effect; and because the specification fails to provide the requisite teachings missing from the prior art, one of skill in the art would have to perform undue experimentation in order to use the invention to obtain clinically relevant therapy, as understood by those of skill in the art (e.g. Alton, Boucher, Rodgers, O'Dea, and Ferrari) in CF treatment.

***Response to Arguments***

Applicant's arguments filed in the Appeal Brief of 3/4/03 have been fully considered but persuasive only in part.

Applicant summarizes the argument at pages 7 and 8 of the Brief, asserting that the Examiner has presented a slanted view of the prior art concentrating on negative aspects of gene therapy and ignoring more favorable reviews, and asserting that the Examiner has created an unreasonable standard for CF therapy by equating "therapy" with "cure".

Applicant argues at page 10 of the Brief that the Examiner has improperly dismissed the Crystal (1999) review because although it was published after the date of filing, it cites many articles published prior to filing of the instant Application. This argument is unpersuasive because Applicant has ignored the rest of the Examiner's argument that stated that the PTO's position was supported by nine pertinent references published both before and after the time of filing, and that Applicant had failed to point out specifically why any teachings of Crystal should be given more weight than those cited in the rejection. In the current rejection, several more post-filing articles have been added to show that, almost 5 years after the time of the invention, success in CF gene therapy has not been obtained.

At pages 10 and 11 of the Brief Applicant argues that the Examiner employed an improper standard for review in applying the standard of CF therapy to claims that require only an increase in susceptibility to viral transduction. This argument is persuasive and is addressed by dropping the species election requirement for claims not explicitly directed to disease treatment, and by limiting the CF gene therapy rejection to claims 53-56, 60, 63-67.

Applicant further argues at page 11 that the Examiner employed an improper standard for review by requiring CF "therapy" to equal "cure". Applicant indicates that a working example demonstrating expression of CFTR in vivo puts the Examiner in the position of having to demonstrate that even a minimal increase in gene expression is of no value to the subject. This argument is unpersuasive for several reasons. First, Applicant has failed to point to any place in the specification wherein an increase in expression of CFTR in vivo is obtained, and the Examiner

was unable to find any. All experiments involving CFTR expression appear to have been carried out in cultured cells in vitro. Second, even if Applicant had shown gene transfer in an animal model, this would not be persuasive evidence of CF treatment. Regarding gene therapy for cystic fibrosis employing animal models, Orkin (1995) stated that although animal investigations are often valuable, it is not always possible to extrapolate directly from animal experiment to human studies" and that "indeed, in some cases, such as cystic fibrosis, cancer, and AIDS, animal models do not satisfactorily mimic the major manifestations of the corresponding human disease" (page 1 lines 30-33). Third, the claims are not limited to any particular amount of treatment correlated with any minimal increase in gene expression. Rather, the claims embrace the entire scope of treatment outcomes ranging from minimal therapeutic outcome to cure, including restoring normal Na ion conductance. Thus, even if the specification had shown some minimal in vivo therapeutic effect, which it does not, it would still fail to enable the full scope of the claims. Fourth, the specification fails to set forth any minimal amount of expression that is correlated with any minimal amount of treatment. Consequently the skilled artisan must turn to the prior art to determine what amount of CFTR expression is required for treatment of CF. The PTO has made of record numerous articles that indicate that at least 6-10% of all lung cells must express CFTR, and possibly many more than that. As noted above, the specification provides no basis for the position that any therapeutic effect can be obtained with less transduction efficiency than 6%, but fails to teach how to achieve this level of transduction in vivo. Applicant asserts at page 17 of the Brief that Figures 5D and 5E of Wang (2000) show greater than 10% transduction. The

Examiner assumes that Applicant refers to Figures 5B and 5D, of Wang (1999) as there is no Figure 5 in Wang (2000) (exhibit I in the Brief) and there is no panel E in Figure 5 of Wang (1999). This assertion is unpersuasive because the Figures supplied are not of sufficiently high resolution to calculate efficiency. It is also not clear that they are representative of the entire transduced area, much less the entire lung, particularly in view of Figure 5C, which appears to show substantially less transduction. It appears that these figures depict isolated transduced foci similar to that observed in Fig. 9 E and F on page 8868 of Johnson et al (1998). Had Johnson cropped the Figure more closely, as in Wang (1999), the apparent transfection efficiency might be as high as 50%, yet the calculated efficiency was only 6.1%. In the absence of any published analysis of the transfection efficiency in Fig. 5 of Wang, Applicant's assertion at page 17 of the brief that greater than 10% of the epithelia is transduced is unpersuasive.

The pre-filing and post-filing art are very clear as to the state of the art of gene therapy of CF. It had not been obtained at the time of filing, and has not been obtained since. The PTO has established through a proper Wands analysis that the scope of the claims is very broad, the state of the art is such that no gene therapy for CF existed before or after the time of filing, the art is highly unpredictable for a variety of well-established reasons, including uncertainty about the appropriate target cells, inability to obtain theoretically adequate delivery and expression, and uncertainty about whether it is sufficient to address Cl ion transport alone, or if Na ion transport is critical to therap. Furthermore it was established that the specification lacked sufficient exemplification and guidance to overcome the shortcomings of the prior art with regard to

enablement of the invention. Accordingly, it is Applicant's burden to establish that the specification teaches how to achieve, in view of the Wands factors discussed above, some level of expression that is therapeutically significant.

With respect to the issues of what defect needs to be rectified (e.g. Na transport or Cl transport) and in what cells, in order to effect therapy, Applicant asserts at page 12 of the Brief that "it may not be necessary to know which cell types to transduce as long as the end result is restoration of chloride transport. This is the general strategy being taken by the field."Applicant offers no support for this assertion. Nowhere has Applicant provided any documentary evidence that "the field" has decided to dismiss the issues of target cells and Na transport. On the contrary, a review of the pre-filing and post filing art shows that these were legitimate concerns expressed by several researchers including at least Rosenfeld and Collins (1996), Wilson (1996), Alton and Geddes (1997), Jiang (1998), Boucher (1999), and O'Dea (2002).

Applicant argues at pages 13 and 14 that the teachings of Zhou provide the only evidence of record regarding the importance of expression in the appropriate cells, and that this evidence indicates that the identity of the cells in which CFTR is expressed may not be important. Zhou teaches transgenic mice, null for mouse CFTR (mCFTR), but carrying one or more human CFTR (hCFTR) alleles expressed in intestinal surface epithelium. Mice lacking mCFTR generally die by the age of 40 days due to intestinal obstruction. Crosses of these null mice with mice transgenic for hCFTR alleles driven by an intestine-specific promoter resulted in increased longevity of mCFTR null mice. Applicant notes that expression of the transgene was not detected in the crypt

epithelium, where mCFTR is normally expressed in the mouse, but was observed instead in the surface epithelium. The conclusion is that ectopic expression of CFTR is sufficient for therapy of CF. The PTO reiterates that this is an invalid conclusion based on non-analogous art. Applicant asserts that in the previous Action the PTO misstated the relevant question which was "whether Applicant was focusing on the correct cell type". In fact it is Applicant who has misstated the question. The question to be considered here is what is the appropriate cell type for **gene therapy** of CF. The animals of Zhou contained a hCFTR allele in every cell, and it is reasonable to expect that these animals contained hCFTR protein in all cells in which the transgene promoter was active. Furthermore, the gene was available for expression throughout the life of the animal beginning in embryogenesis. This could explain why the chloride transport rate observed in the hybrid mice was sufficient to prevent intestinal obstruction. See Zhou, page 1708, column 2, lines 5-11. Because Zhou uses a transgenic animal and does not address issues of gene delivery to a whole organism, Zhou is not dispositive of whether or not delivery to the appropriate cell type is immaterial to methods of gene therapy of CF. Neither the specification nor the prior art enables one to use gene therapy methods to achieve delivery to 100% of cells and obtain expression throughout the development of the animal beginning in embryonic stages, therefore one cannot reasonably conclude that the results of Zhou show that the identity of target cells is immaterial to gene therapy of CF. It is abundantly clear from the references cited above, *e.g.* Rosenfeld and Collins (1996), Wilson (1996), Alton and Geddes (1997), Jiang (1998), Boucher (1999), and O'Dea (2002), that those of skill in the art considered the identity of cells to be transfected, the

amount of gene expression obtained, the pattern of gene expression obtained, and the defect to be corrected to be relevant issues deserving of further research, and that due in part to these factors, gene therapy for cystic fibrosis did not exist before, at, or after the time of filing. Thus, contrary to the assertion at page 14 of the Brief, the burden of establishing enablement of CF gene therapy has clearly been shifted to Applicant.

At pages 14 Applicant argues that because Johnson teaches that adding 6-10% normal cells to a population of CF cells in vitro corrects the chloride ion defect, Boucher's position that it may be necessary to transduce of 100% of cells is invalid. Further, at pages 16 and 17 Applicant addresses the opinion of Boucher that it is likely that the percentage of epithelial cells requiring functional correction may well exceed 10%, stating that this "is not accepted as the fundamental issue facing gene therapy." This statement is unsupported. The subsequent discussion at pages 16 and 17 provides no evidence that less than 10% of cells would need to be transfected to provide therapeutic evidence, and does not address in any way the reason for Boucher's conclusion, i.e. that it is likely that the number of gap junctions *in vivo* is less than that in the *in vitro* monolayer model on which the estimation of 6-10% is based. Because there is reason to doubt that more transduction may be required in vivo than in vitro, and because Applicant has presented no evidence to the contrary, the argument is unpersuasive.

Applicant goes on to argue that even thought the invention may ultimately find no use in treating humans it may be a significant and useful contribution to the art. The Examiner has no doubt that Applicant has made a significant and useful contribution to the art of viral vector

delivery to epithelial cells. However, the rejected claims require treatment of CF and embrace a broad scope including restoration of Na ion transport and a cure of the disease. The foregoing Wands analysis demonstrates that the specification has not adequately enabled this use of the invention.

Applicant further argues that the demonstration of in vitro Cl ion transport defect correction and in vivo reporter gene delivery is all that is required to enable the invention, relying for support on *Cross v. Iizuka*.. However, in *Cross v. Iizuka*, the court did not find that the "how to use requirement" for in vivo therapy can be satisfied by demonstration of pharmacological activity in vitro. The enablement issue in *Cross v. Iizuka* was strictly limited to determining in vitro efficacy. The court found that the disclosure was enabling for determining $I_{C50}$ in an in vitro microsomal system, and clearly stated that the issue under consideration was pharmacological activity in vitro and not therapeutic use in vivo. See 224 USPQ at page 748.

> The Board found that there was sufficient credible evidence that one skilled in the art, without the exercise of inventive skill or undue experimentation, could determine the $IC_{50}$ dosage level for the imidazole derivatives of the phantom count in the microsome environment. Cf. Bundy, id., 209 USPQ at 51. We do not believe the Board erred in arriving at this conclusion. **This is not a case such as In re Gardner, 427 F.2d 786, 166 USPQ 138 (1970), where the CCPA held that the applicant's disclosure was nonenabling because inventive skill and undue experimentation would be required to discover appropriate dosages for humans, i.e., a therapeutic use. In the instant case, we are confronted with a pharmacological activity or practical utility, not a therapeutic use.**

Emphasis added. Furthermore, even if *Cross v. Iizuka* did support the correlation between in vivo and in vitro results, it is not analogous to the instant invention. In *Cross v. Iizuka*, the issue was enablement of inhibitors of thrombaxane synthetase, not gene therapy. The standard and practice for testing thrombaxane synthetase inhibitors was well settled at the time of filing, and the

correlation between structural and functional of the inhibitors was well characterized, so unpredictability was not as great an issue as in the instant case. Here, the Office has established that the art of CF gene therapy is highly unpredictable, and there is uncertainty in the art as to what is the appropriate end point for assays of therapy. It is also noted that Applicant's reliance for support in Limberis (2002) (Exhibit G in the Brief) is misplaced. Limberis used a permeabilizing agent that was not disclosed in the instant invention, lysophosphatidylcholine (LPC), to enhance lentivirus-mediated transfer of CFTR to mouse nasal airway epithelium in vivo, to correct Cl ion transport. Limberis noted that LPC might have augmented gene transfer in a variety of ways that would be specific to LPC, including its mucolytic properties, its tendency to reduce cilial beat, and its improvement in vector particle deposition. See page 1968, column 2. For these reasons Limberis goes beyond the teachings of the instant disclosure and cannot be used as evidence of enablement at the time the invention was filed, because developments occurring after the filing date of an application are of no significance regarding what one skilled in the art believed as of that filing date. See for example, *in re Wright*, 27 USPQ2d 1510, 1514 (Fed. Cir. 1993). Interestingly, Limberis also notes that there is not necessarily any correlation between in vivo and in vitro gene transfer results. See page 1968 column 1, second paragraph.

Finally, at pages 17 and 18, Applicant considers the issue of vector access to the epithelium, particularly as affected by mucus, or the absence of mucus in infant and young children. Applicant asserts that the Examiner has not met the burden of establishing that an unreasonable number of cystic fibrosis patients have an unreasonable amount of mucus. Applicant

also asserts that Karp et al (2002) shows that an in vitro model of differentiated human airway epithelia has beating cilia and secretes salt water and mucus. The inference is that in vitro models showing transduction of human CF airway primary cultures are predictive of what would happen in vitro. This argument is unpersuasive because Applicant has not shown that the cultures in the instant application were grown under the same conditions, and have the same characteristics, as those in Karp. Applicant discloses at page 75 of the specification that the methods of Yamaya (1992) and Zabner (1996) were used to grow human cells. At the paragraph bridging pages 122 and 123, Karp states that the methods described were adapted from those used by Yamaya (1992) in such a way that longevity and transepithelial transport were increased. So, there is reason to doubt that the secretion characteristics of the cells used in the specification were different than those used in the instant invention. In any event, the argument is unpersuasive because it fails to provide sufficient evidence to show that the specification is enabling for the full scope of the claims, i.e. for a method of gene therapy for cystic fibrosis for the reasons summarized above. As noted above at length, the specification fails to provide adequate guidance with respect to the target cells that be transduced with CFTR expression vectors in order to obtain therapeutic benefit in CF treatments, how many of these cells must be transfected, or what level of expression must be obtained to effect therapy, and fails to teach how to obtain even the minimum transduction efficiency postulated to be sufficient for therapy. For these reasons the rejection is maintained.

***Claim Rejections - 35 USC § 102***

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless -
>
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.
>
> (e) the invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who has fulfilled the requirements of paragraphs (1), (2), and (4) of section 371(c) of this title before the invention thereof by the applicant for patent.

At the outset, it should be noted that claims 1-8 and 10-25, while directed to a method of increasing susceptibility to viral infection, do not recite any step of viral infection, and require only treatment of cells with a permeabilizing agent, whereby an increase in transepithelial permeability increases susceptibility to viral infection. For this reason, references which teach increasing transepithelial permeability by treatment with a permeabilizing agent are considered to anticipate the claims embracing the permeabilization agent taught. See for example rejections over Kleeberger, Debs, Jongeneel, Marano, Tomita, Zegarra-Moran, Meza, McEwan, Wong, Saldias, Rischardson, Hashimoto, and Li, below.

Claims 48 and 49 are directed to methods of redistributing viral receptors in epithelial tissue in which the only active step is "increasing the transepithelial permeability of said epithelial

tissue". For this reason any method which results in increasing transepithelial permeability is considered to anticipate the claims.

Claims 1-5, 13, 14, 26-28, 30, and 48-50 are rejected under 35 U.S.C. 102(a) as being anticipated by Kaplan et al (Human Gene Therapy (1998) 9(10): 1469-1479).

Kaplan teaches a method of increasing replication deficient adenoviral transduction and expression of a human transgene in alveoli, bronchial, and tracheal epithelium by nasal instillation of adenoviruses and polylysine. See entire abstract, page 1470, column 2, first and second full paragraphs; page 1472, column 1, lines 2-18; Fig. 4, panel A on page 1474; and Fig. 5 on page 1474.

Claims 1-5, 22, 23, 48, and 49 are rejected under 35 U.S.C. 102(b) as being anticipated by Kleeberger et al (Applied Phys. (1992) 72(2): 670-676).

Kleeberger teaches a method of increasing permeability in airway epithelia by forcing rats to breath ozone gas. See abstract, and page 671, column 1, paragraph 2.

Kleeberger is silent as to whether these treatments increase the susceptibility of cells to viral infection, however this outcome is taken to be inherent because Kleeberger teaches the required method step of contacting cells with a tissue permeabilizing agent, ozone, and a subsequent increase in permeability.

Claims 1, 2, 4, 6, 7, 26, 27, 30, and 48-52 are rejected under 35 U.S.C. 102(a) as being anticipated by Johnson et al (J. Virol. (1998) 72(11): 8861-8872).

Johnson teaches a method of delivering to tracheal cells in vivo a replication defective retroviral vector, and causing expression of a non-viral reporter gene. Epithelial tight junction permeability and proliferation were increased by treatment with sulfur dioxide gas prior to infection. See abstract, and paragraph bridging pages 8869 and 8870. Because sulfur dioxide causes an increase in epithelial tight junction permeability and stimulates proliferation it is both a permeabilizing agent and a proliferative factor within the meaning of the claims.

Claims 1, 2, 13, 14, 26- 30, 32, 33, 36, 37, 48, 50, 53-56, 60, and 63-65 are rejected under 35 U.S.C. 102(e) as being anticipated by Welsh et al (US Patent 5,962,429, issued 10/5/99).

Welsh teaches a method of providing cystic fibrosis transmembrane conductance regulator to airway epithelial cells of a cystic fibrosis patient comprising combining cationic polypeptide polymers such as poly-L-lysine or histone with adenoviral particles containing a transgene encoding cystic fibrosis transmembrane conductance regulator, wherein said transgene is expressed and a functional chloride channel is produced in the airway epithelial cells of said patient. See claim 13. Addition of polycation to the adenovirus results in increased infection. See Fig. 13, and column 21, lines 50-59. The complexes may be delivered as an aerosol. See column 9, lines 40-43 and column 10, lines 44-48. The complexes may be applied topically, i.e. by

instillation or lavage. See column 10, lines 5-9 and 44-48. While it is not clear that the permeability of the diseased tissue was increased in this method, the simplest explanation of the results is that infection efficiency was improved by addition of the polycations. This can be construed as an increase in permeability as the entrance of virus into the epithelial cells involves traversal of an epithelial cell membrane. The office does not have the facilities for examining and comparing Applicant's product with the product of the prior art in order to establish that the product of the prior art does not possess the same material, structural and functional characteristics of the claimed product. In the absence of evidence to the contrary, the burden is upon the applicant to prove that the claimed products are functionally different than those taught by the prior art and to establish patentable differences. See Ex parte Phillips, 28 USPQ 1302, 1303 (BPAI 1993), In re Best, 562 F.2d 1252, 195 USPQ 430 (CCPA 1977) and Ex parte Gray, 10 USPQ2d 1922, 1923 (BPAI 1989).

Claims 1, 2, 4, 13, 26, 27, 30-33, 35, 37-39, 41-44, 46-50, and 52 are rejected under 35 U.S.C. 102(b) as being anticipated by Olsen et al (Nucl. Acids Res. (1993) 21(3): 663-669), as evidenced by Weisz et al (US Patent 5,658,894, issued 8/19/97), and Quinn et al (J. Cell. Phys (1996) 168(1):34-41.

Olsen teaches a method of replication-deficient retroviral transduction of a cystic fibrosis tracheal epithelial cell line. See abstract. Transduction efficiency was improved when retrovirus infection was carried out in the presence of the polycationic polypeptide protamine. See page

664, column 2, third full paragraph. The method resulted in the expression of the polypeptide IL-2. Claims 37-39, 41-44 are included in the rejection because transfections were carried out in a solution comprising retroviruses, fetal bovine serum, and protamine. See page 664, column 1, last sentence of second full paragraph, and third full paragraph; and page 664, column 2, first sentence of third full paragraph. Viruses supernatants from PA317 packaging cells grown in medium containing 10% fetal bovine serum were added to cell cultures in the presence of protamine. So, the culture medium in the infection procedure contained retroviruses, fetal bovine serum, and protamine. Fetal bovine serum contains proliferative factors. See column 14, lines 64 and 65 of Weisz. Thus, the liquid medium in which the infection was carried out is a composition comprising packaged retroviruses, proliferative factors, and a permeabilization factor (protamine). Because the medium is liquid it is considered to be suitable for aerosol application as required by claim 38. Because the polycation and the fetal bovine serum were delivered directly to a cultured epithelium in a dish, administration is considered to be topical as required by claims 35, and 37. Quinn provides evidence that the retroviral vector used, LISN, is replication deficient.

Claims 1, 5, 18, 26, 27, 30, 37, and 48-50 are rejected under 35 U.S.C. 102(b) as being anticipated by Katkin et al (Human Gene Therapy 1997 3(9): 75-779).

Katkin teaches a method of infecting alveolar epithelia with a replication defective adenoviral vector comprising a lacZ gene. The virus was delivered topically to the lung epithelium. The delivery composition comprised 10% glycerol, a permeabilizing agent. See

abstract; sentence bridging columns 1 and 2 on page 172; and second full paragraph of column 2 on page 172.

Claims 1, 6-8, 24, 25, 35, 48, and 49 are rejected under 35 U.S.C. 102(b) as being anticipated by Marano et al (Biochem. Biophys. Res. Comm. (195) 209(2): 669-676).

Marano teaches a method of increasing transepithelial permeability by treatment of epithelia with TNF- alpha. See abstract and Fig. 5 on page 674. The cells of Marano are considered to be in the presence of a proliferative agent as required by claims 6-8, because they are cultured in 10% fetal bovine serum which contains a variety of growth factors. Administration of the proliferative factor is considered to be topical because fetal bovine serum it is applied directly to the cells.

Claims 1, 7, 8, 13, 26-31, 35, 37, and 48-52 are rejected under 35 U.S.C. 102(b) as being anticipated by Cornetta et al (J. Virol. Methods (1989) 23(2): 187-194).

Cornetta teaches a method of increasing replication-deficient retroviral transduction of epithelial cells by exposure to the polycationic polypeptide protamine. The retrovirus contained a human ADA enzyme gene. Claim 35 is included in the rejection because the direct addition to cells of fetal calf serum, containing proliferative factors, is considered to be topical application. Claim 51 is included in the rejection because the cell culture contained fetal calf serum which causes proliferation of cells. See abstract and paragraph bridging pages 18 and 189.

Claims 1, 7, 34, 36, 37, 48, and 49 are rejected under 35 U.S.C. 102(b) as being anticipated by Debs et al (J. Immunol. 1988 140(10): 3482-3488).

Debs teaches a method of aerosol delivery of the permeabilization factor TNF-alpha to the epithelium of rat lungs. Because the aerosol delivery is local to the lungs and contacts the epithelium directly, this is considered to be topical application. Redistribution of retroviral receptors is considered to be inherent.

Claims 1, 10-12, 48, 49, and 68 are rejected under 35 U.S.C. 102(b) as being anticipated by Jongeneel et al (Nucl. Acids Res. (1980) 8(7): 1661-1673).

Jongeneel teaches a method of permeabilizing epithelial cells by shock with a hypotonic solution comprising EGTA. See abstract and sentence bridging pages 1662 and 1663.

Claims 1, 11, 12, 48, and 49 are rejected under 35 U.S.C. 102(b) as being anticipated by Tomita (JOURNAL OF PHARMACEUTICAL SCIENCES, (1996 Jun) 85 (6) 608-11).

Tomita teaches a method of permeabilizing epithelial cells by exposure to EDTA. See abstract.

Claims 1, 11, 12, 48, and 49 are rejected under 35 U.S.C. 102(b) as being anticipated by Zegarra-Moran et al (BRITISH JOURNAL OF PHARMACOLOGY, (1995 Mar) 114 (5) 1052-6).

Zegarra-Moran teaches a method of permeabilizing epithelial cells by exposure to BAPTA. See abstract.

Claims 1, 11, 12, 16, 17, 48, and 49 are rejected under 35 U.S.C. 102(b) as being anticipated by Meza et al (JOURNAL OF CELLULAR BIOCHEMISTRY, (1982) 18 (4) 407-21).

Meza teaches a method of permeabilizing epithelial cells by exposure to cytochalasin B or EGTA. See abstract.

Claims 1, 13, 14, 48, and 49 are rejected under 35 U.S.C. 102(b) as being anticipated by McEwan et al (Biochim. Biophys. Acta (1993) 1148(1): 51-60).

McEwan teaches a method of increasing paracellular permeability of epithelial tissue by treatment with polylysine. See abstract.

Claims 1, 13, 14, 26, 27, 30, 32, 33, and 48-50 are rejected under 35 U.S.C. 102(b) as being anticipated by Arcasoy et al (Gene Therapy (1997) 4(1): 32-38).

Arcasoy teaches a method of increasing adenoviral transduction and expression of a transgene in diseased epithelial cells in vitro by exposing the epithelial cells to polylysine or polylysine. See abstract. The adenovirus is replication deficient. See paragraph bridging pages 36

and 37. The cells are an immortalized cystic fibrosis airway epithelial line expressing a defective CFTR. See abstract.

Claims 1, 15, 48, and 49 are rejected under 35 U.S.C. 102(b) as being anticipated by Wong et al (J. Cell Biol. (1997) 1363(2): 399-409).

Wong teaches a method of increasing paracellular transepithelial permeability by treatment of epithelia with an occludin peptide. See abstract.

Claims 1, 17, 48, and 49 are rejected under 35 U.S.C. 102(b) as being anticipated by Yap et al (Exp. Cell Res. (1995) 218(2): 540-550).

Yap teaches a method of increasing paracellular transepithelial permeability by treatment of epithelia with colchicine. See abstract.

Claims 1, 18, 48, and 49 are rejected under 35 U.S.C. 102(b) as being anticipated by Richardson et al (Lab. Invest. (1976) 35(4): 307-314).

Richardson teaches a method of increasing transepithelial permeability of tracheal epithelium by treatment of the epithelium with ether. See abstract.

Claims 1, 19, 20, 48, and 49 are are rejected under 35 U.S.C. 102(b) as being anticipated by Hashimoto et al (Biochim. Biophys. Acta (1997) 1323(2): 281-290).

Hashimoto teaches a method of increasing epithelial tight junction permeability by treatment of the epithelium with the neurotransmitter capsianoside. See abstract.

Claims 1, 21, 48, and 49 are rejected under 35 U.S.C. 102(b) as being anticipated by Li et al (BIOCHIMICA ET BIOPHYSICA ACTA, (1990 Dec 14) 1030 (2) 297-300).

Li teaches a method of increasing epithelial tight junction permeability by treatment of the epithelium with FCCP. See abstract.

Claims 1, 22, 23, 48, and 49 are rejected under 35 U.S.C. 102(b) as being anticipated by Welsh et al (J. Clin. Invest. (1985) 76: 1155-1168).

Welsh teaches a method of increasing paracellular transepithelial permeability by treatment of epithelia hydrogen peroxide. See abstract.

Claims 38, 39, 41-44, 46, and 47 are rejected under 35 U.S.C. 102(b) as being anticipated by Flasshove et al (Blood (1995) 85(2): 566-574), as evidenced by Weisz et al (US Patent 5,658,894, issued 8/19/97).

Flasshove teaches a composition comprising a solution of replication deficient retroviruses, fetal bovine serum, and the permeabilization agents protamine and IL-1. See page 567, column 2, lines 11-15 and page 568, column 1, lines 3-6. Fetal bovine serum contains proliferative factors. See column 14, lines 64 and 65 of Weisz. Although the solution comprises a

suspension of cells, it is still suitable for aerosol or topical delivery, absent evidence to the contrary.

Claims 68 and 69 are rejected under 35 U.S.C. 102(b) as being anticipated by Wunderlich et al (ARCHIVES OF VIROLOGY, (1982) 73 (2) 171-83).

Wunderlich teaches a suspension of viral particles in a hypotonic solution consisting of 1 mM EGTA, 5 mM Tris-HCl. See page 173, third full paragraph.

***Conclusion***

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner(s) should be directed to Richard Schnizer, whose telephone number is 703-306-5441. The examiner can normally be reached Monday through Friday between the hours of 6:20 AM and 3:50 PM. The examiner is off on alternate Fridays, but is sometimes in the office anyway.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, John Leguyader, can be reached at 703-308-0447. The FAX numbers for art unit 1632 are 703-308-4242, and 703-305-3014. Additionally correspondence can be transmitted to the following RIGHTFAX numbers: 703-872-9306 for correspondence before final rejection, and 703-872-9307 for correspondence after final rejection.

Inquiries of a general nature or relating to the status of the application should be directed to the Patent Analyst Trina Turner whose telephone number is 703-305-3413.

DAVE T. NGUYEN
PRIMARY EXAMINER

Richard Schnizer, Ph.D.